

Amendments to the Specification

Please replace paragraph [0010.0] with the following:

[0010.1] ~~Based on all these observations, it is believed that introducing PBP_s into cotton fiber will increase the fiber strength, water absorption, thermal characteristics and chemical reactivity~~
~~What is required for the commercial viability of protein-based polymers is a cost of production that would begin to rival that of petroleum-based polymers. The potential to do so resides in low cost bioproduction. We have recently demonstrated a dramatic hyperexpression of an elastin protein-based polymer, (Gly-Val-Gly-Val-Pro)_n or poly(GVGVP), which is a parent polymer for a diverse set of polymers that exhibit inverse temperature transitions of hydrophobic folding, and assembly as the temperature is raised through a transition range and which can exist in hydrogel, elastic, and plastic states. Electron micrographs revealed formation of inclusion bodies in *E. coli* cells occupying up to 80-90% of the cell volume under optimal growth conditions (3a). The beauty of this approach is the lack of any need for extraneous sequences for the purposes of purification (4) or adequate expression. The usual strategy for expression of a foreign protein or protein-based polymer in an organism such as *E. coli* anticipates that the foreign protein will be injurious to the organism. Accordingly, the transformed cells are grown up to an appropriate stage before expression of the foreign protein is begun and expression is generally considered viable for only a few hours. The situation is quite different for the elastic protein-based polymer considered here. This may result in part due to the extraordinary biocompatibility exhibited by (GVGVP)_n and its related polymers. The elastic protein-based polymer, (GVGVP)_n and its γ -irradiation crosslinked matrix as well as related polymers and matrices appear to be ignored by a range of animal cells and by tissues of the whole animal (5-7). This chapter describes in detail methodologies to accomplish hyperexpression of a protein based polymer in *E. coli*.~~

Please insert the following new paragraphs between paragraphs [0010.1] and [0011] bridging pages 7 and 8:

[0010.2] Construction of a synthetic protein-based polymer gene: As an illustration of an uninduced hyper-expression of a protein-based polymer in *E. coli*, we have chosen a gene encoding 121 repeats of the elastomeric pentapeptide:

Pro gly val gly val pro (GVGVP)8 gly val gly val pro gly val
cggatCCA GGC GTT GGT-----CCA GGT GTT Ggatccg
BamH1 PfIM1 PfIM1 BamH1

[0010.3] Genes for the tricosapeptides GVGVP GVGFP GEGFP GVGVP GVGFP GFGFP and GVGVP GVGFP GDGFP GVGVP GVGFP GFGFP, analogous to compounds LXII and LIX, respectively, were constructed using synthetic oligonucleotides. The double-stranded DNA sequence of these genes with the corresponding amino acid sequence is the following (Equation 1):

BamH-1 PfIM-1
5' -GAGGATCCAGGC~~G~~TTGGGTACCGGGTGTGGCTCCCG
3' -ctccttaggtccgcaaacccatggccacaacccgaaggc

G V \ G V P G V G F P

GGTGAMGGTTCCCGGGCGTGGTGTGccg
ccacikcCAAAGGGCCCGCAACCACAGGC

G X G F P G V G V P
ggtgtaggcttcgggttcggatccccaggcgttggatccag-3'
CCACATCCGAAAGGCCAAAGCCTAAGGTCCGAAACCTAGGTC-5'

G V G F P G F G F P

[0010.4] Fig. 1. Amino acid sequence and flanking restriction endonuclease sites of the basic polymer building block coding for (GVGVP)₁₀. Using synthetic oligonucleotides and PCR, ((GVGVP)₁₀ was amplified with flanking BamH1 and PfIM1 ends and the 121-mer gene was inserted into pUC118 as a BamH1 fragment. For expression under control of the T7 polymerase

gene promoter, a 121-mer gene was created by concatenation of the PflM1 10-mer fragment with terminal cloning adaptors and subsequently inserted into the expression vector pET-11d.

[0010.4] GVGVP. This gene, (GVGVP)₁₂₁, was constructed by ligase concatenation of DNA sequence encoding (GVGVP)₁₀ and isolation of a concatemer having 12 repeats of this monomer gene plus an additional C-terminal (GVGVP) sequence encoded by a 3' cloning adaptor (10a). The gene encoding (GVGVP)₁₀ was synthesized and cloned into a multipurpose cloning plasmid from which it was then excised by digestion at flanking sites with the restriction endonuclease PflM1 (Fig. 1). A substantial amount of the PflM1 gene fragment was purified and self-ligated in the presence of limited amounts of synthetic double-stranded oligonucleotide adapters that provided the additional restriction sites needed for cloning the resulting concatemers. PflM1 cleaves at its recognition site in the DNA to leave two single-stranded extensions that are not self-complementary (i.e., nonpalindromic) but are only complementary to each other; therefore, proper translational polarity is maintained by head-to-tail tandem coupling of the monomer gene units by ligase during the concatenation reaction.

Please replace paragraph [0014] with the following:

[0014] ~~In contrast we attempt here to express a protein polymer and not a polyester. PBP~~ used in our study are expressed from a single synthetic gene that can easily be altered to increase the fiber strength, water absorption, thermal properties, elasticity and dye binding capacity of cotton fiber by changing the amino acid composition. We attempt to accomplish this using a gene encoding GVGVP₁₂₁ (SEQ. ID. NO.3); this gene has been expressed at high levels in bacteria (Figure 1; Daniell et al., 1997) and tobacco plants (Figure 2; Daniell and Guda, 1997). Transgenic tobacco plants expressing this PBP grew, flowered and produced seeds normally (Zhang et al., 1996). However, this gene has not previously been expressed in cotton fibers. In stably transformed tobacco plants a 1.8 kbp EG-120mer polymer gene fragment was found to be

integrated into the tobacco nuclear genome. A 1.8 kbp EG-120mer polymer gene transcript was observed in Northern blots. Gels stained with CuCl₂ show the presence of polymer and Western blots confirm the identity of the polymer protein (Zhang et al., 1995, 1996). Even though lower levels of expression were observed in cultured tobacco cells (Zhang et al., 1995) and some transgenic plants in the F0 generation (probably due to the position effect and heterozygous nature, Zhang et al., 1996), higher levels of polymer expression were observed in tobacco cells (see Figure 2); this is a good indication of a PBP express (Daniell, 1995; Daniell and Guda, 1997). The transgenic tobacco plants expressing the PBP grew, flowered and produced seeds normally (Zhang et al., 1996). Physiological and ultrastructural studies reveal that transgenic tobacco plants expressing PBP are similar to control untransformed plants.